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14. ABSTRACT Incorporating synthetic polypeptides with intrinsic secondary structures into side chains of brush-like polymers may facilitate interesting physical properties of these materials, as the entanglement and association behaviors of the polypeptide chains significantly differ from those of flexible polymer coils. This pilot study aimed to correlate the physical properties of polypeptide-grafted brush polymers with the structure and the interactions of grafted polypeptide chains. In the 9-month project, we have synthesized a small library of brush polymers with grafted polypeptides of specific compositions and molecular weights, by integrating ring-opening metathesis					
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Report Title

STIR: A Pilot Study on the Bulk Properties and Morphology of Polypeptide-Grafted Brush Polymers

ABSTRACT

Incorporating synthetic polypeptides with intrinsic secondary structures into side chains of brush-like polymers may facilitate interesting physical properties of these materials, as the entanglement and association behaviors of the polypeptide chains significantly differ from those of flexible polymer coils. This pilot study aimed to correlate the physical properties of polypeptide-grafted brush polymers with the structure and the interactions of grafted polypeptide chains. In the 9-month project, we have synthesized a small library of brush polymers with grafted polypeptides of specific compositions and molecular weights, by integrating ring-opening metathesis polymerization (ROMP) and ring-opening polymerization of amino acid N-carboxyanhydrides (NCA). The structures and association behaviors of the grafted polypeptide chains in these brush polymers have been characterized by FTIR, NMR and X-ray scattering methods and correlated with the architecture of the macromolecules. We found that grafted polypeptides with long helical structures facilitate the intermolecular association of the brush-like polymers, and allow for the formation of robust films in which the molecular orientation can be induced by mechanical stretching or thermal annealing. The molecular nature of the entanglement behaviors found in these unique brush polymers may be further elucidated by studying the viscoelastic properties of these materials.

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Hongwei Xia	0.55	
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STIR: A Pilot Study on the Bulk Properties and Morphology of Polypeptide-Grafted Brush Polymers

Dr. Yao Lin, Assistant Professor of Polymer and Chemistry, Department of Connecticut, Storrs

A. SUMMARY OF THE MOST IMPORTANT RESULTS.

Incorporating synthetic polypeptides with intrinsic secondary structures into side chains of brush-like polymers may facilitate interesting physical properties of these materials, as the entanglement and association behaviors of the polypeptide chains significantly differ from those of flexible polymer coils. However, brush polymers with grafted polypeptides are rarely investigated due to the challenge of synthesizing these materials with controlled compositions and structures. The structure-property relationship of these materials is largely unexplored. This pilot study aimed to correlate the physical properties of polypeptide-grafted brush polymers with the structure and the interactions of grafted polypeptide chains. In the 9-month project, we have synthesized a small library of brush polymers with grafted polypeptides of specific compositions and molecular weights, by integrating ring-opening metathesis polymerization (ROMP) and ring-opening polymerization of amino acid *N*-carboxyanhydrides (NCA). The structures and association behaviors of the grafted polypeptide chains in these brush polymers have been characterized by FTIR, NMR and X-ray scattering methods and correlated with the architecture of the macromolecules. We found that grafted polypeptides with long helical structures facilitate the intermolecular association of the brush-like polymers, and allow for the formation of robust films in which the molecular orientation can be induced by mechanical stretching or thermal annealing. The molecular nature of the entanglement behaviors found in these unique brush polymers may be further elucidated by studying the viscoelastic properties of these materials.

B. STATEMENT OF THE PROBLEM STUDIED.

B.1. Synthesize a limited number of polypeptide-grafted brush polymers with specific compositions based on integrated ROMP/NCA polymerization.

Previously, we have demonstrated an effective strategy of integrating ROMP and ROP of NCAs to make brush-like polymers containing grafted polypeptide chains with controlled compositions. We applied the method to synthesize six polynorbornene-*g*-poly(γ -benzyl-L-glutamate) (PN-*g*-PBLG) brush polymers with specific number (*x*) and DPs (*n*) of grafted polypeptide chains (e.g., PN_{*x*}-*g*-PBLG_{*n*}, where *x*=10, 20 and *n*=12, 50, 100). The controlled ROMP and NCA polymerizations gave the expected MW and narrow MWD (**Table 1**). Monomodal GPC distribution patterns were observed with the brush-like polymers containing different numbers of grafted chains and the grafted chains of different lengths (**Figure 1**). Prior studies have showed that in bulk, linear PBLG forms the α -helical secondary structure when its DP is larger than 14, and forms the β -sheet secondary structure when the DP is less than 14. Using the small library of PN-*g*-PBLG brush polymer we synthesized, we can carry out the studies to examine how the association of these unique brush polymers depends on the secondary structures, the numbers

and the lengths of the grafted polypeptide chains. In addition, four polynorbornene-*g*-poly(ϵ -benzyloxycarbonyl-*L*-lysine) brush polymers (PN_x-*g*-PZLL_n, where *x*=10 and *n*=12, 50, 70, 100) were also synthesized to examine whether the knowledge obtained from the study on PN-*g*-PBLG is applicable to other types of polypeptides that also form well-defined helical secondary structures.

Entry	Sample	Mn _{expect} ($\times 10^5$ g/mol)	Mn _{calc} ($\times 10^5$ g/mol)	PDI
1	PN ₁₀ - <i>g</i> -PBLG ₁₂	0.303	0.343	1.11
2	PN ₁₀ - <i>g</i> -PBLG ₅₀	1.196	1.125	1.06
3	PN ₁₀ - <i>g</i> -PBLG ₁₀₀	2.371	2.310	1.06
4	PN ₂₀ - <i>g</i> -PBLG ₁₂	0.605	0.686	1.1
5	PN ₂₀ - <i>g</i> -PBLG ₅₀	2.391	2.258	1.06
6	PN ₂₀ - <i>g</i> -PBLG ₁₀₀	4.741	4.482	1.05

Table 1. Synthesis of Polynorbornene-*g*-poly(γ -benzyl-*L*-glutamate) brush polymers with different numbers and DPs of grafted chains.

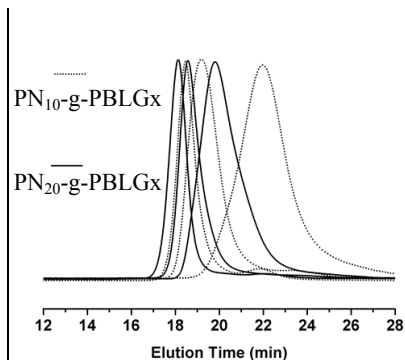


Figure 1. GPC overlay of six PN_x-*g*-PBLG_n samples in DMF (*x*=10, 20 and *n*=12, 50, 100).

B.2. Investigate the film-forming property of the polypeptide-grafted brush polymers and identify the polypeptide secondary structures in the brush polymers by Fourier transform infrared spectroscopy (FTIR).

We first investigated the film-forming properties of the polypeptide-grafted brush polymers. The polymers were casted from chloroform onto a polytetrafluoroethylene (PTFE) plate at room temperature (25 °C) and vacuum dried. The films of the brush polymers with short polypeptide grafts (e.g., PN₁₀-*g*-PBLG₁₂ and PN₂₀-*g*-PBLG₁₂) were found to be very brittle. Cracks were formed in these films after they were removed from the glass slide (e.g., PN₁₀-*g*-PBLG₁₂ film in the top panel of **Figure 2A**), indicating the absence of strong intermolecular chain association. On the contrary, freestanding films can be readily prepared from the brush polymers with polypeptide grafts with a DP of 50 or 100. Moderate fracture strength and bending strength were found in these polymers (e.g., PN₁₀-*g*-PBLG₁₀₀ film in the bottom panel of **Figure 2A**), suggesting that the long grafted polypeptides considerably enhance the intermolecular associations in the brush polymer materials. The glass transition temperatures of all PN-*g*-PBLG brush polymers were found to be around 20 °C, as determined by differential scanning calorimetry (DSC) (**Figure 2B**). The thermal gravimetric analysis (TGA) shows the polymers are stable up to a temperature of 250 °C. Annealing the samples at 150 °C under vacuum further improves the bending strength of the brush polymers with high molecular weights. Similar behaviors have also been found in the films prepared from the PN-*g*-PZLL brush polymers.

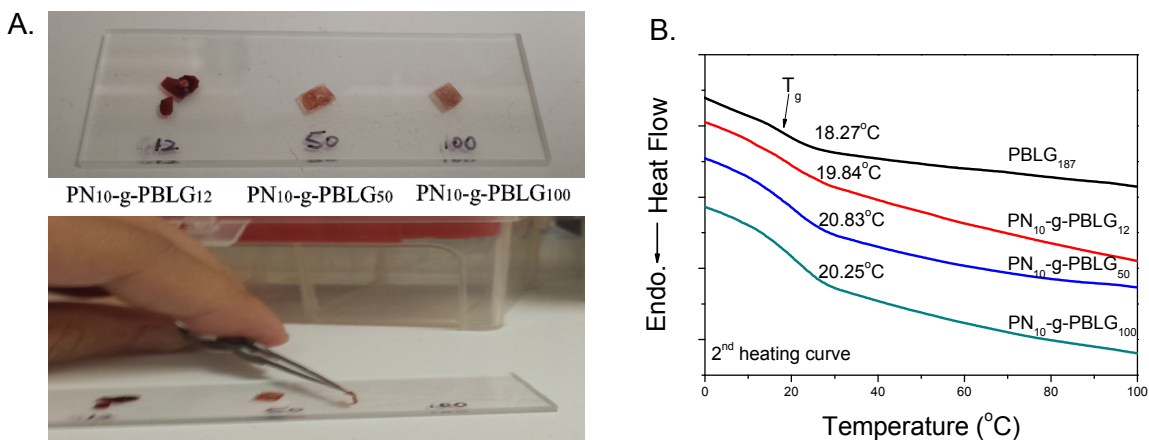


Figure 2. (A) Film-forming properties and (B) and the glass transition temperatures of the polypeptide-grafted brush polymers with different side chain lengths.

We then used Fourier transform infrared spectroscopy (FTIR) to distinguish the polypeptide conformational structures in the PN-g-PBLG brush polymer thin films. Different polypeptide secondary structures (e.g., α -helix, β -sheet and random coil) in the brush polymers were readily resolved by IR spectroscopy (**Figure 3**). Our result shows that, while PN10-g-PBLG12 and PN20-g-PBLG12 possess a significant portion of β -sheet and random coil structures, helical structure dominates in all the other brush polymers with longer PBLG grafts (e.g., DP=50 or 100), as clearly identified from the amide I bands around 1650 cm^{-1} . The number of grafts chains per brush polymers (x) does not affect much on the secondary structures. The films, however, are not oriented enough to conduct the polarized IR experiments.

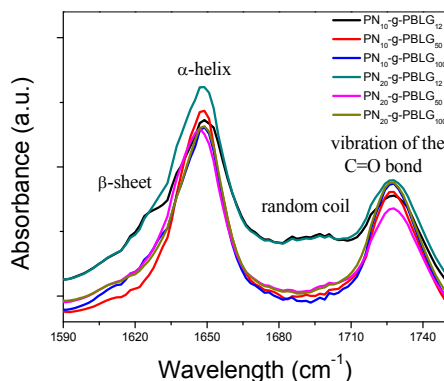


Figure 3. FTIR spectra of PN-g-PBLG brush polymers with different numbers and DPs of grafted chains.

B.3. Study the morphology and the linear dynamic mechanical property of the polypeptide-grafted brush polymers.

We investigated the morphologies of brush polymers bearing specific grafted polypeptides by wide angle and small angle X-ray scattering (WAXS and SAXS). **Figure 4** shows the WAXS profiles from the six PN-g-PBLG brush polymers with different numbers and DPs of grafted chains. Except PN10-g-PBLG12 and PN20-g-PBLG12, the WAXS pattern displays strong scattering with relative positions that reflect hexagonal packing of α -helices. The ordering of helical side-chains in the film can be further improved by thermal or solvent annealing (not

shown here). The SAXS experiments, however, did not provide much information on the average separation distance between individual brush polymers. Therefore, we cannot assert the extent of interdigitation between the brush polymers. The neutron scattering experiments on the brush polymers with labeled backbone should be carried out in the future to obtain this information. Qualitatively, the longer grafted polypeptides appear to have more interpenetration and ordering in relative to the shorter chains. Increasing the density of grafted chains (e.g., in PN20-g-PBLG_n) made it more difficult for the polypeptides to facilitate interdigitation effectively.

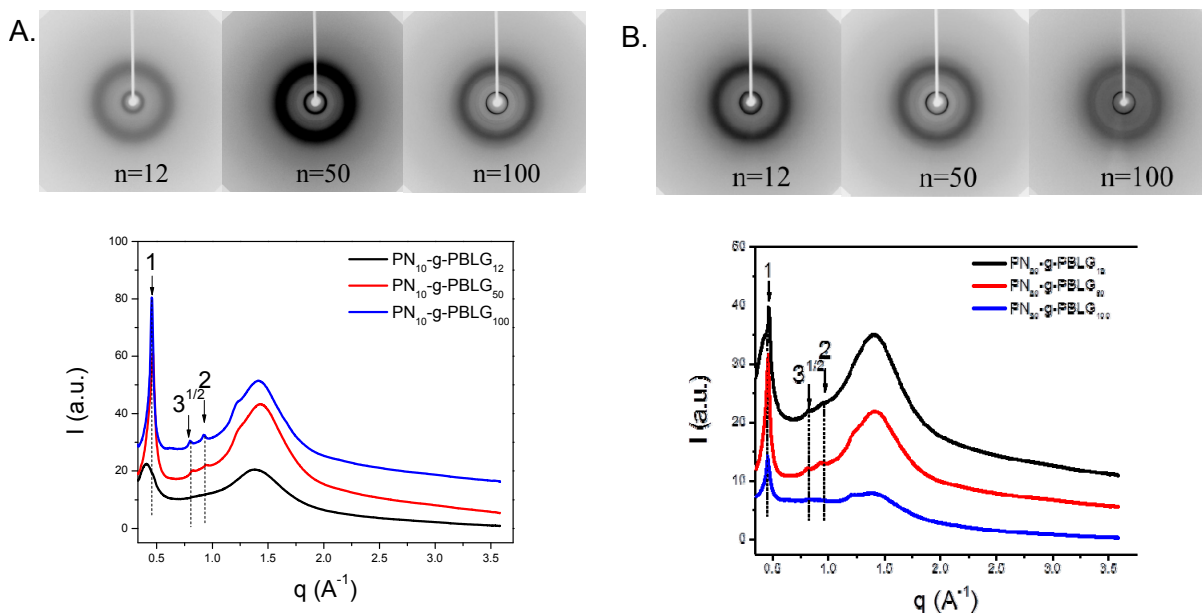


Figure 4. Wide-angle X-ray images and scattering profiles of (A) PN10-g-PBLG_n thin films, where n=12, 50 and 100, and (B) PN10-g-PBLG_n thin films, where n=12, 50 and 100.

As the brush polymers with long helical chains have good mechanical strength as a result of the intermolecular associations, we tested whether the molecular orientation can be further induced by mechanical stretching. Thin fibers were made from PN10-g-PBLG100 and PN20-g-PBLG100, by stretching and drying the viscous samples containing small amount of solvent (Figure 5A-B). The WAXS patterns from the stretched polymer fibers display strong equatorial reflections with relative positions that reflect hexagonal packing of α -helices (e.g., PN10-g-PBLG100 in Figure 5B). The meridian reflection corresponds to the helical pitch (0.54 nm) of a normal α -helical conformation of 18 residues in 5 turns with a repeat unit of 2.7 nm, i.e., an 18/5 helix. In contrast, the molecular alignments cannot be further induced in PN_x-g-PBLG50 or PN_x-g-PBLG10 samples by mechanical stretching. We also extended the scattering studies on the different types of helical polymers (e.g., PN-g-PZLL) and found similar behaviors (data not shown). The brush-polymers containing long helical polypeptides are thus most promising materials that may possess desire physical properties, due to their unique intermolecular association behaviors.

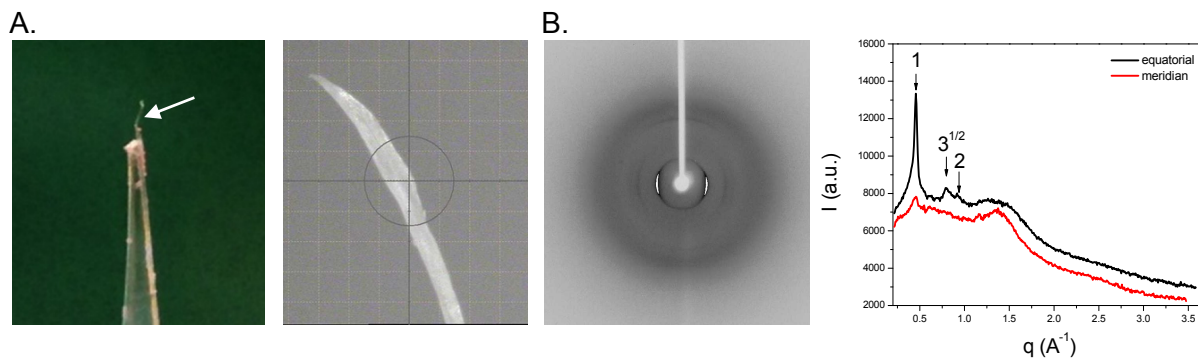


Figure 5. (A) The images of a stretched fiber of PN10-g-PBLG100, and (B) the wide-angle X-ray image and the scattering profile of the fiber.

Finally, we carried out some preliminary experiments on testing the linear dynamic mechanical property of PN-g-PBLG brush polymers using the rheometer with parallel plate geometry. Due to the limited amount of samples we have synthesized for this pilot study (150~200 mg per sample), we had difficulty in obtaining reproducible data to construct a master curve of G' and G'' by the time-temperature principle. Larger amount of samples (0.5~1 g per sample) need to be synthesized in the future study to determine the dynamic shear moduli properties of these brush polymers. These experiments will eventually elucidate how the intermolecular chain entanglements depend on the type and length of the polypeptides in the brush polymers, and establish a connection with their morphology and film forming properties we obtained.